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Transcervical artificial insemination in sheep: effects of a new transcervical artificial insemination instrument and traversing the cervix on pregnancy and lambing rates☆

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Abstract

Cervical anatomy limits the use of transcervical intrauterine artificial insemination (TC AI) in sheep. We have developed an instrument to cope atraumatically with the cervix; although this instrument has not affected fertilization rate or pregnancy rate through Day 3, the effects on sperm transport and pregnancy after Day 3 are not known. The objective of the present study was to determine whether our TC AI instrument affected sperm transport, pregnancy rates, or lambing rate. In Experiment 1, ewes were assigned to two treatments: TC AI using the new TC AI instrument ($n = 10$) or AI via laparotomy using a laparoscopic AI instrument ($n = 10$). Twenty hours after artificial insemination, the uterine horns and oviducts were recovered and flushed to collect spermatozoa. Sperm transport did not differ ($P > 0.05$) between the two treatments. In Experiment 2, ewes were assigned to three treatments: TC AI using the new TC AI instrument + sham intrauterine AI via laparotomy ($n = 29$); sham TC AI + intrauterine AI via laparotomy using a laparoscopic AI instrument ($n = 29$); and sham TC AI + intrauterine AI via laparotomy using the new TC AI instrument ($n = 30$). On Day 14 after AI, uteri were collected and flushed to recover blastocysts. Transcervical deposition of semen reduced ($P < 0.05$) Day 14 pregnancy rate

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(17.2% versus 61%), but intrauterine deposition of semen using the TC AI instrument via midventral laparotomy increased ($P < 0.05$) Day 14 pregnancy rate (76.6% versus 44.8%). In Experiment 3, ewes were assigned to two treatments: sham cervical manipulation ($n = 40$) or cervical manipulation to mimic TC AI ($n = 40$). Immediately after treatment, each ewe was mated with a ram and watched until the ram mounted and ejaculated into the ewe. Treatment did not affect Day 30 or 50 pregnancy rate (67.5 and 66.2%, respectively), determined ultrasonically, or lambing rate (62.5%). The differences between Days 30 and 50 pregnancy rates and lambing rate were not significant. In Experiment 4, ewes were assigned to two treatments: TC AI ($n = 99$) or laparoscopic AI ($n = 99$). Transcervical AI reduced ($P < 0.01$) Day 30 (TC AI versus laparoscopic AI; 5.0% versus 46.0%) and Day 50 pregnancy rates (4.0% versus 41.0%), determined ultrasonically, and lambing rate (4.0% versus 41.0%). Although the TC AI procedure significantly reduced pregnancy and lambing rates, large numbers of spermatozoa deposited at natural insemination seemed to compensate. Because our TC AI procedure has all but eliminated any visual evidence of trauma, and because the procedure does not seem to affect sperm transport or embryonal survival until Day 3, we speculate that cervical manipulation associated with TC AI may activate pathways that interrupt pregnancy between Days 3 and 14.

Keywords: Artificial insemination; Cervix; Sheep

1. Introduction

The lack of nonsurgical artificial insemination (AI) and embryo transfer (ET) procedures for sheep severely limits the use of these technologies. Surgical AI and ET (i.e., laparascopic or via midventral laparotomy) are effective, but they are costly, time consuming, require technical proficiency, limit the number of times ewes can be used, and require anesthesia [1]. However, in ewes the tortuous nature of the cervix restricts the passage of transcervical (TC) AI and ET catheters [2,3], effectively preventing TC AI and ET and mandating surgical AI and ET. There are two major barriers in sheep: size and shape of the external cervical os and eccentric nature of the cervical canal [4–7]. Therefore, effective transcervical AI and ET procedures for sheep must include an atraumatic method of traversing the cervix.

There are three obvious methods for reducing the physical effects of the ovine cervix: physically (e.g., attaching a hemostat to the external cervical os and retracting the cervix to align the cervical os and decrease obstructions to the uterine lumen) [7], chemically (e.g., dilating the cervix with PGE_2 or oxytocin) [8–12], or mechanically (e.g., by designing appropriate TC AI and ET equipment to overcome the physical difficulties associated with the ovine cervix) [12]. Currently, most TC AI and ET equipment for sheep resembles AI and ET equipment for cattle, and the equipment is usually too rigid and too large in diameter for the sheep cervix. These instruments typically cause bruising and tearing of the cervix, possibly causing the release of spermicidal or embryocidal compounds and therefore decreasing fertility [13].

In an attempt to overcome the problems associated with TC AI (i.e., trauma induced as the instrument is manipulated through the cervix and into the uterus), a new TC AI instrument was developed. Neither this instrument nor passing it through the cervix affected semen characteristics, fertilization, or pregnancy rate through Day 3 [12].

However, whether this instrument or passage of the instrument through the cervix affects sperm transport or pregnancy rates after Day 3 is not known. Thus, the objective of the present study was to determine whether this TC AI instrument and/or using this instrument to perform TC AI affected sperm transport or pregnancy and lambing rates.

2. Materials and methods

2.1. General

All procedures involving animals were in compliance with USDA, ARS guidelines and United States Sheep Experiment Station (USSES), Institutional Animal Care and Use Committee guidelines. Unless stated otherwise, all sheep used for these experiments were maintained at the USSES, fed adequate diets, and given free access to water.

Four hundred and twenty ewes (Experiment 1 included Rambouillet; Experiments 2–4 included Rambouillet × Targhee × Polypay crossbreds) were allotted to the four experiments (Table 1). In each experiment, age of ewe and parity were balanced across treatments. Nulliparous ewes were not used for any of the experiments. Experiment 1 was conducted in early February 2003 and Experiments 2–4 were conducted in November 2002. The USSES lambs once per year, beginning early in March and ending early in May. Therefore, postpartum interval was uniform across treatments in each experiment. For a full description of the sheep and distribution across experiments, see Table 1.

2.2. Estrus synchronization

Progestogenated pessaries (Veramix; 60 mg of medroxyprogesterone acetate; Pharmacia Animal Health, Orangeville, Ont., Canada) for 12 days, $PGF_{2\alpha}$ (15 mg; Lutalyse; Pharmacia Animal Health, Kalamazoo, MI, USA) treatment on Day 6, and 400 IU of eCG (Sioux Biochemical, Sioux City, IA, USA) at the time of pessary removal were used to synchronize estrus during the annual breeding season [12]. Ewes were checked for estrus 48 h after pessary removal, using vasectomized rams. Ewes standing firmly to be mounted were considered to be in estrus. If ewes were not observed in estrus after pessary removal, they were not used for these experiments. In Experiment 2, 90 ewes were assigned to three treatments ($n = 30$ ewes/treatment group), but one ewe was not detected in estrus, resulting

Table 1
Characteristics[a] of ewes used in Experiments 1–4

Experiment	Total assigned	Total used	Breed	Age (year)	Parity	Interval postpartum (day)
1	20	20	Rambouillet	6.4 ± 0.5	4.2 ± 0.7	318 ± 22
2	90	88	Crossbred[b]	4.2 ± 1.3	2.1 ± 1.0	204 ± 31
3	80	80	Crossbred[b]	4.5 ± 1.3	2.8 ± 0.6	200 ± 23
4	200	198	Crossbred[b]	5.1 ± 2.3	3.4 ± 1.3	206 ± 41

[a] Age, parity, and interval data are means ± S.E. (no significant difference among treatments).
[b] Rambouillet × Targhee × Polypay crosses.

in 29, rather than 30, ewes receiving treatment 2. In Experiment 4, 200 ewes were assigned to two treatments ($n = 100$ ewes/treatment group), but one ewe was not detected in estrus, resulting in 99, rather than 100, ewes receiving Treatment 2. Across all four experiments, the estrus synchronization procedure, based on detection of estrus, was successful in 418 of 420 sheep. All analysis of data is based on ewes that were detected in estrus and consequently received treatments.

2.3. Transcervical AI catheter

The design, testing, and calibration of the TC AI catheter used for this study has been described in detail [12]. The design of the catheter (Fig. 1) permits semen to be deposited in the uterine horn, rather than in the caudal portion of the uterine body. The flexibility of the catheter enables atraumatic passage through the cervix and into the uterus, without kinking the catheter and blocking the flow of semen through it. Another design feature allows one to

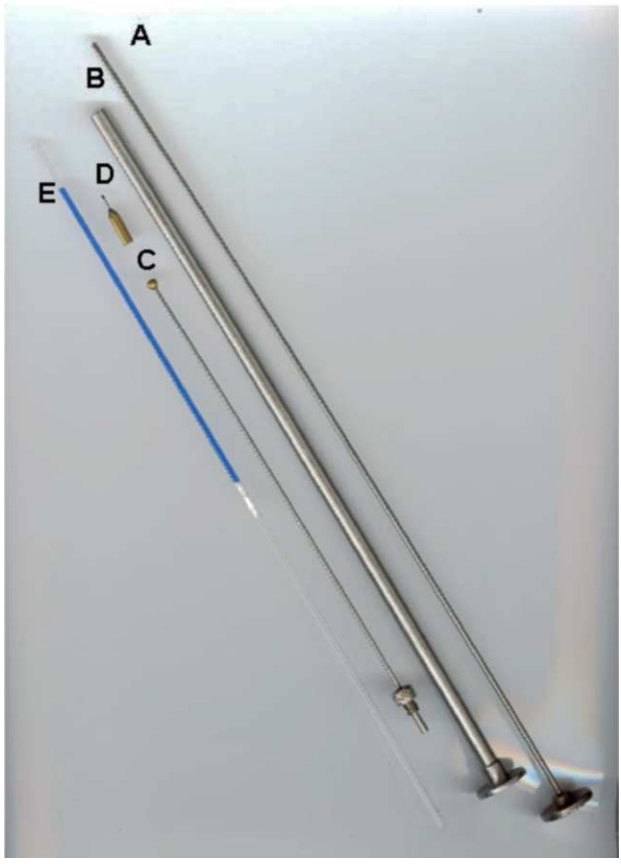

Fig. 1. Artificial insemination equipment used during Experiments 1–4. (A) Plunger for retroload AI instrument, (B) retroload AI instrument, (C) TC AI tip, (D) laparoscopic AI needle, (E) 1 cm^3 semen straw.

use transrectal ultrasonography to visualize passage of the catheter through the cervix, into the uterine body, and into a uterine horn [10]. With practice, and based on the characteristics of the vaginal, cervical, and uterine tissues detected with the TC AI instrument, one can distinguish the parts of the reproductive tract without transrectal ultrasonography [10]. Because of the required length, diameter, and flexibility of the catheter, a retroload AI gun was designed (Fig. 1) to hold a 1 cm^3 semen straw, so that an adequate volume of fluid can be used to compensate for the void volume of the catheter and make delivery of small volumes of semen possible. This additional fluid does not seem to alter ovum fertilization rates [12].

2.4. Semen preparation

Semen for AI was collected with an artificial vagina from crossbred rams of known fertility and diluted with an egg yolk citrate extender [1] or an aloe vera extender (Experiments 2–4) [14]. Diluted semen from the same five rams in Experiment 1 and the same nine rams in Experiments 2–4 was combined on each day of AI to overcome ram variation within the experiment. Rams had individual sperm motilities >40%. At the time of AI, motility and forward progressive movement were assessed. The insemination dose contained from 200×10^6 to 350×10^6 spermatozoa per 0.2 mL. The number of spermatozoa used was considerably greater than some recommendations [1]. However, we used large numbers of spermatozoa to reduce the likelihood that small numbers would limit pregnancy rates and to prevent spermatozoa from becoming too dilute after addition of excess extender associated with loading the semen straw to accommodate the new TC AI catheter ([12], R.G. Saacke, personal communication, W.M.C. Maxwell, personal communication).

2.5. Transcervical AI

In preparation for TC AI, each ewe was restrained in dorsal recumbency in a Poldenvale Commodore cradle (Premier Sheep Supplies, Washington, IA, USA), and the perineal area was scrubbed with antiseptic soap and rinsed thoroughly [12]. Dry gauze sponges were used to remove excess water and antiseptic. A vaginoscope (tubular speculum) was coated with obstetric lubricant that did not contain a spermicide. The vaginoscope was inserted into the vagina and pushed against the tissue surrounding the cervix to help center the external cervical os. A cattle AI catheter was placed into the folds of tissue surrounding the external cervical os to position the cervix in the vaginoscope, and then the catheter was removed. The cattle AI catheter was used only as a positioning tool; it was not inserted into the cervix, and it was not used to ''open'' the external cervical os or to dilate the cervix. Immediately after the cervical os was centered in the vaginoscope, the brass tip of the TC AI catheter was placed at the cervical os, and the catheter was manipulated through the cervix. After moving the catheter through the cervix, a 1 cm^3 semen straw containing 0.2 mL of diluted semen (loaded last to be expelled first), a small air bubble, and 0.6 mL of extender (to compensate for void volume), was inserted into the retroload AI gun. The semen was then expelled through the TC AI catheter into the uterus. The catheter was removed slowly, and the vaginoscope was removed. The ewe was returned to a standing

position and removed from the cradle. Because of the potential for causing cervical damage if duration of TC AI (i.e., traversing the cervix and delivering the semen and extender) was >2 min, a ewe was removed from the experiment if the procedure exceeded a 2 min limit. Typically, we are unable to traverse the cervix without causing overt damage in approximately 2% of the parous ewes, and this was the case for this study.

Two ewes were removed from experiments because it took >2 min to traverse the cervix. Initially, 90 ewes were assigned to Experiment 2; however, it took >2 min to traverse the cervix of one ewe assigned to Treatment 1; so 29, rather than 30, ewes received Treatment 1. In Experiment 4, it took >2 min to traverse the cervix of one ewe assigned to Treatment 1; so 99, rather than 100, ewes received Treatment 1. All analysis of data is based on the number of ewes that received the treatments.

2.6. Midventral laparotomy AI

For AI via midventral laparotomy, all ewes were anesthetized with sodium pentobarbital (Vedco, St. Joseph, MO, USA; 60 mg/mL; administered i.v., until ewes reached a surgical plane of anesthesia) and restrained in dorsal recumbency on a surgery table. The abdominal area was shorn, scrubbed with antiseptic soap, and rinsed. The uterine horns were exposed through an abdominal incision, and, depending on experiment and treatment, a laparoscopic AI instrument (Fig. 1) or the TC AI catheter was used for AI. For laparotomy AI with the laparoscopic AI instrument, a needle attached to the instrument was inserted into the uterine horn just anterior to the uterine body, and 0.2 mL of diluted semen was injected through the needle followed by 0.6 mL of extender, in 0.2 mL increments, to equal the volume of extender used to compensate for the void volume of the TC AI instrument.

For laparotomy AI with the TC AI catheter, one tip of a mosquito hemostatic forceps was used to create a 2–3 mm diameter incision through the myometrium and endometrium. The tip of the TC AI catheter was inserted through the incision and into the uterine lumen. A 1 cm^3 semen straw containing 0.2 mL of diluted semen (loaded last to be expelled first), a small air bubble, and 0.6 mL of extender was inserted into the retroload AI gun. The semen was expelled through the TC AI catheter and into the uterine lumen. The TC AI catheter was removed from the uterine horn. After AI, the uterus was returned to the abdominal cavity as quickly as possible, the laparotomy was sutured closed, and the ewe was moved to a clean, dry recovery pen.

2.7. Transrectal ultrasonography

For Experiments 3 and 4, an Aloka 500 V instrument equipped with either a 7.5 or 3.5 MHz linear array transducer (Corometrics Medical Systems, Inc., Wallingford, CT, USA) was used to diagnose pregnancy at 30 and 50 days, respectively, after AI.

2.8. Experiment 1

This experiment was conducted to confirm TC deposition of semen into the uterus and to determine whether our new TC AI catheter affected sperm transport. Ewes were assigned to two treatments: TC AI using the new TC AI instrument + sham intrauterine AI via

laparotomy ($n = 10$) or sham TC AI + AI via laparotomy using a laparoscopic AI instrument ($n = 10$).

For the sham TC AI, a vaginoscope was lubricated, inserted into the vagina, left in place for approximately 30 s, and then removed. The time to perform TC AI was recorded for each ewe; recording this information is a standard part of our procedure. For the sham laparotomy AI, the uterine horns were exposed through the abdominal incision and returned immediately to the abdominal cavity, and the laparotomy was sutured closed.

At 48–52 h after pessary removal, ewes were restrained in dorsal recumbency in a Poldenvale Commodore cradle and were either subjected to a sham TC AI procedure (30 s) or to TC AI (mean time to perform TC AI was 33 ± 8 s). Immediately after that procedure, ewes were either artificially inseminated via midventral laparotomy, using either a laparoscopic AI instrument, or the sham procedure was performed.

Uterine and oviductal flushings were collected 20 h after AI. Ewes were stunned with a captive bolt pistol and exsanguinated. The uterus with the mesometrium, mesosalpinx, mesovarium, ovaries, and oviducts intact, and a portion of the cervix were removed postmortem. Next, the uterine horns and oviducts were flushed individually with sterile, isotonic saline. Flushings were centrifuged for 10 min at 2000 × g, and the supernatant was removed. The pellet was resuspended in 1 mL of saline containing 0.1% sodium azide and centrifuged for 10 min at 17,000 × g. The final pellet was resuspended in 3 mL of saline containing 0.1% sodium azide and stored at 4 °C until the sperm concentration was determined. To improve determination of sperm concentration, samples were sonicated (21 kHz for four 30-s bursts) to disrupt cellular debris in the flushings. With this procedure, spermatozoa lost their tails, but sperm heads remained intact. Sperm concentration was determined with a hemocytometer using standard procedures [15], and concentration was used to calculate the total number of spermatozoa in each flushing.

2.9. Experiment 2

This experiment was conducted to determine whether our new TC AI catheter affected Day 14 pregnancy rate and whether the process of moving this catheter through the cervix to perform TC intrauterine AI would affect Day 14 pregnancy rate. Ewes were assigned to one of three randomized treatments: TC AI using the new TC AI catheter + sham intrauterine AI via laparotomy ($n = 29$); sham TC AI + intrauterine AI via laparotomy using a laparoscopic AI instrument ($n = 29$); and sham TC AI + intrauterine AI via laparotomy using the new TC AI catheter ($n = 30$). Sham TC AI and sham AI via midventral laporotomy were performed as described in Experiment 1. Mean time to perform TC AI in this experiment was 42 ± 18 s.

Using one of the three methods, ewes were artificially inseminated 48–52 h after pessary removal. All ewes were restrained in dorsal recumbency in a Poldenvale Commodore cradle and were either subjected to a sham TC AI procedure or to TC AI. Immediately after that procedure, ewes were either artificially inseminated via midventral laparotomy, using either a laparoscopic AI instrument or the TC AI catheter, or the sham procedure was performed.

Embryos were collected 14 days after AI. Ewes were stunned with a captive bolt pistol and exsanguinated. The uterus with the mesometrium, mesosalpinx, mesovarium, ovaries, and oviducts intact, and a portion of the cervix were removed postmortem. The cervix was

incised and examined for any evidence of damage (e.g., bruising, edema, or tearing). No damage or abnormalities were observed 14 days after AI in any of the groups. Next, the uterine contents were flushed out with sterile, isotonic saline (20 mL). The flushings were examined macroscopically and microscopically. Ewes were considered to be pregnant if an elongated blastocyst was present in the uterine flushings. Pregnancy rates were calculated as $(\text{number of ewes with embryos}/\text{number of ewes}) \times 100$.

2.10. Experiment 3

This experiment was conducted to determine whether movement of our new TC AI catheter through the cervix affected Days 30 and 50 pregnancy rate or lambing rate. Ewes were assigned to one of two randomized treatments: sham cervical manipulation ($n = 40$) or cervical manipulation to mimic TC AI ($n = 40$). Mean time to perform TC AI in this experiment was 21 ± 9 s. During sham or cervical manipulation, each ewe was restrained in dorsal recumbency in a Poldenvale Commodore cradle. For the sham procedure, a vaginoscope was lubricated, inserted into the vagina, left in place for approximately 30 s, and then removed. For cervical manipulation to mimic TC AI, the TC AI procedure was performed, but neither semen nor diluent were injected into the uterus. At 48–52 h after pessary removal, ewes received one of the two treatments. Immediately after treatment, each ewe was mated with a ram and watched until the ram mounted and ejaculated into the ewe.

At 30 and 50 days after breeding, transrectal ultrasonography was used to determine pregnancy rates. Pregnancy rates were calculated as (number of ewes pregnant on Day 30 or on 50/number of ewes bred) $\times$ 100. Ewes were maintained through lambing to determine lambing rate: (number of ewes lambing/number of ewes bred) $\times$ 100.

2.11. Experiment 4

This experiment was conducted to determine the efficacy our new TC AI catheter when used for TC AI versus the efficacy of laparoscopic AI. Efficacy was evaluated at Days 30 and 50 of pregnancy and at lambing. Ewes were assigned to one of two treatment groups: TC AI ($n = 99$) or laparoscopic AI ($n = 99$). At 48–52 h after pessary removal, ewes were artificially inseminated transcervically or laparoscopically [1]. At 30 and 50 days after AI, transrectal ultrasonography was used to determine pregnancy rates. Pregnancy rates and lambing rate were calculated as described in Section 2.9.

2.12. Statistical analyses

The GLM procedures of SAS (SAS Inst. Inc., Cary, NC, USA) were used to determine the effects of treatment, location (i.e., segment of the reproductive tract), and treatment $\times$ location interaction on sperm numbers. Hartley's fmax test [16] indicated that the variances were heterogeneous, so sperm numbers were transformed to natural logarithms [15]. When F-tests were significant, Duncan's procedure was used to compare the means. All inferences were based on the analysis of transformed data.

The GLM procedures of SAS were used to analyze the data from Experiments 2–4. Because ewe was the experimental unit, pregnancy rates were evaluated on a per-ewe basis.

Table 2
Number of spermatozoa in the uterine horn and oviducts after TC AI or AI via midventral laparotomy using a new TC AI catheter or a laparoscopic AI instrument

Variable[a]	Intrauterine semen deposition[b,c]		
	Transcervical	Laparotomy	S.E.M.
Left oviduct	2.97	1.54	1.97
Right oviduct	3.21	1.48	2.14
Left uterine horn	2.94	3.23	2.02
Right uterine horn	2.86	3.51	2.78

[a] Each variable was evaluated on a per ewe basis.
[b] Treatment 1: TC intrauterine AI using a new TC AI catheter + sham intrauterine AI via laparotomy ($n = 10$); Treatment 2: sham TC AI + intrauterine AI via laparotomy using a laparoscopic AI instrument ($n = 10$). Semen was deposited intrauterine either transcervically (Treatment 1) or via laparotomy (Treatment 2).
[c] Means are the natural logarithm of sperm numbers. Because the variances were heterogenous, the natural logarithms of sperm numbers were used for data analysis.

Even though Chi-square methods are often used to analyze categorical data, we used GLM procedures (i.e., least squares methods) because conclusions derived from Chi-square and analysis of variance, using least squares methods, are usually the same [12,17,18].

3. Results

3.1. Experiment 1

Transcervical intrauterine deposition of semen did not alter sperm transport ($P > 0.05$) into the uterus or the oviducts, when compared with intrauterine deposition of semen via midventral laparotomy (Table 2).

3.2. Experiment 2

Transcervical deposition of semen reduced ($P < 0.05$) Day 14 pregnancy rate (Table 3); whereas, intrauterine deposition of semen using the TC AI instrument via midventral laparotomy increased ($P < 0.05$) Day 14 pregnancy rate (Table 3).

3.3. Experiment 3

There was no significant difference between the two treatment groups for pregnancy rate on Days 30 and 50 or for lambing rate (Table 4).

3.4. Experiment 4

Transcervical AI reduced Days 30 and 50 pregnancy rates and lambing rate (Table 5). Even though the Day 50 pregnancy rate was five percentage points less than the Day 30 pregnancy rate for the laparoscopic AI group, the difference was not significant. The Day 50 pregnancy rate was the same as the lambing rate.

Table 3
Day 14 pregnancy rate of ewes after TC AI or AI via midventral laparotomy using a new TC AI catheter or a laparoscopic AI instrument

Variable[a]	Semen deposition[b]		Instrument for surgical deposition[c]	
	Transcervical	Surgical	Transcervical	Laparoscopic
Pregnancy (%)	17.2[d]	61.0[e]	76.6[f]	44.8[g]

(d, e) Means with different superscripts differ ($P < 0.05$). (f, g) Means with different superscripts differ ($P < 0.05$).

[a] Variables were evaluated on a per ewe basis.

[b] Treatment 1: TC intrauterine AI using a new TC AI catheter + sham intrauterine AI via laparotomy ($n = 29$); Treatment 2: sham TC AI + intrauterine AI via laparotomy using a laparoscopic AI instrument ($n = 29$); and Treatment 3: sham TC AI + intrauterine AI via laparotomy using the new TC AI instrument ($n = 30$). Semen was deposited either transcervically (Treatment 1) or surgically (Treatments 2 and 3).

[c] For surgical AI, semen was deposited either with the new TC AI catheter (Treatment 2) or with the laparoscopic AI instrument (Treatment 3).

Table 4
Days 30 and 50 pregnancy rates and lambing rate after sham cervical manipulation or cervical manipulation immediately before natural insemination

Rate[a]	Treatment[b]	
	Sham cervical manipulation	Cervical manipulation
Day 30 pregnancy (%)[c]	67.5	67.5
Day 50 pregnancy (%)	67.5	65.0
Lambing (%)	65.0	60.0

[a] Each variable was evaluated on a per ewe basis.

[b] Sham cervical manipulation $n = 40$; cervical manipulation $n = 40$.

[c] Treatment did not affect ($P > 0.05$) Day 30 and 50 pregnancy rates or lambing rates.

Table 5
Days 30 and 50 pregnancy rates and lambing rate after transcervical AI or laparoscopic AI ($n = 198$ ewes)

Rates[a]	Treatment[b]	
	Transcervical AI	Laparoscopic AI
Day 30 pregnancy (%)[c]	5.0[c]	46.0[d]
Day 50 pregnancy (%)[d]	4.0[c]	41.0[d]
Lambing (%)	4.0[c]	41.0[d]

(c, d) Means with different superscripts in the same row differ ($P < 0.01$).

[a] Each variable was evaluated on a per ewe basis.

[b] Transcervical AI, $n = 99$; laparoscopic AI, $n = 99$.

4. Discussion

Our TC AI procedure did not affect sperm transport into the oviducts. Performing our TC AI procedure, but without depositing semen or diluent into the uterus, just before natural insemination, to allow a ram to deposit large numbers of spermatozoa into the vagina, did

not reduce Day 30 or 50 pregnancy rates or lambing rate. However, using our TC AI procedure to deposit a ''fixed'' number, which was considerably less than a typical ram would deposit, of fresh, diluted spermatozoa directly into the uterus reduced Days 14, 30, and 50 pregnancy rates and lambing rate, even though our TC AI procedure has not affected semen characteristics, sperm transport, fertilization rate or pregnancy rate through Day 3 [12]. This seems to indicate that the TC AI procedure per se interferes with pregnancy sometime between Days 3 and 14 and that large numbers of spermatozoa ejaculated into the vagina can compensate for the deleterious effects of this procedure.

Previous studies indicated that anatomical features of the ovine cervix hindered TC AI [19]. However, those studies did not incorporate atraumatic methods for managing the physiological and anatomical barriers that the ovine cervix imposes. Results of the present and previous studies indicated that our TC AI catheter permitted intrauterine deposition of semen either transcervically (i.e., nonsurgically) or surgically, via laparotomy and a small incision through the uterine horn, without producing damage that is detectable 3, 12, or 14 days later [10,12]. In fact, using our TC AI instrument for AI via laparotomy increased Day 14 pregnancy rate compared with using a laparoscopic instrument for AI via laparotomy. The increased pregnancy rate and lambing rates after surgical AI indicated that the TC AI catheter itself does not reduce fertilization rate or the establishment of pregnancy.

The process of manipulating an AI catheter through the cervix has already been linked to reductions in pregnancy and lambing rates [12–14]. However, the cause is unclear. We hypothesize that cervical trauma or downstream manifestations of vaginal and cervical stimulation induce the release of a spermicidal compound [13]. However, because our previous research indicated that TC AI does not reduce fertilization rate [12], one would not expect a true spermicidal compound to have caused the reduction in pregnancy and lambing rates in this study. Nevertheless, one might speculate that effects of vaginal and cervical manipulation initiated at the time of manipulation but that did not manifest for several days could alter embryonic development and induce embryonal mortality, which may be evidence of an embryocidal effect. More specifically, the negative effects of cervical manipulation may be amplified between the time of semen deposition and fertilization, but the effects may not be manifested until after fertilization. For example, manual examination of the cervix in late pregnant sheep increased uterine releases of $PGF_{2\alpha}$ [20], and transcervical uterine flushes during the luteal-phase of estradiol 17-β-treated cattle increased uterine luminal $PGF_{2\alpha}$ content [21]. The $PGF_{2\alpha}$ increases in both of these examples were after periods of progesterone priming and estradiol stimulation, somewhat similar to the conditions at the time of TC AI in the present study.

Prostaglandin $F_{2\alpha}$ has a variety of well-known effects on immune function [22,23]. Indeed, $PGF_{2\alpha}$ is chemotactic to neutrophils in vitro [22], although $PGF_{2\alpha}$ has not been shown to have chemotactic properties in the uterus, and $PGF_{2\alpha}$ can up-regulate uterine immune functions. Even though the time of AI is too early for $PGF_{2\alpha}$ to affect luteal function, increased $PGF_{2\alpha}$ could affect ovulation or CL development.

In pigs, animals in which semen is deposited directly into the uterus at mating, leukocytes typically migrate into the uterus in large numbers during estrus, without disrupting fertilization and embryonic development [24]. However, rams deposit semen into the vagina, rather than directly into the uterus, and intrauterine deposition of semen, compared with diluent, increased the number of polymorphonuclear leucocytes in the uterine lumen [25].

Thus, because transcervical deposition of semen is not a natural process in sheep, transcervical AI may activate pathways that are associated with uterine immune defenses.

Perhaps cervical stimulation associated with TC AI increases uterine $PGF_{2\alpha}$ production, and uterine $PGF_{2\alpha}$ then enhances the migration of neutrophils into the uterus and stimulates processes that activate neutrophils. These events may then change the uterine immune environment and render the uterus unsuitable for embryonic development. Indeed, in postpartum dairy cattle, pregnancy rates decreased significantly as the number of neutrophils in uterine flushings before AI increased [26]. In addition, our embryo transfer experiments with sheep seem to support the idea that delayed effects of cervical manipulation associated with transcervical embryo transfer interfere with embryonic development [10]. After transcervical embryo transfer on Day 6, embryos continue to develop, and their gross morphology on Day 12 appears consistent with that of Day 12, elongated blastocysts [10]. However, Day 14 blastocysts were fragmented, and their development appeared to have ceased [10]. The data from postpartum dairy cows and from our sheep embryo transfer experiments seem to indicate that uterine events that are not typical for the stage of the reproductive cycle can interfere with pregnancy.

Allowing rams to deposit large numbers of spermatozoa at the external cervical os seemed to have compensated for the negative effects of the cervical manipulation component of the TC AI procedure. Whether spermatozoa that were deposited into the vagina and had traveled through the cervix were ''protected'' in some way, as has been proposed for cattle (R.G. Saacke, personal communication), or whether a portion of the large number of spermatozoa that were deposited naturally near the cervical os arrived at the site of fertilization undamaged and able to produce embryos that can develop into lambs, is not known. Whatever the mechanism, transcervical intrauterine AI with relatively small numbers of spermatozoa, compared with natural insemination, in this study had a catastrophic effect on pregnancy and lambing rates.

In conclusion, neither our new TC AI catheter for sheep nor passage of the catheter through the cervix affected sperm transport or Days 14, 30 or 50 pregnancy rates or lambing rates when large numbers of spermatozoa were inseminated. By contrast, using the TC AI instrument to deposit relatively small numbers of spermatozoa into the uterus dramatically reduced pregnancy and lambing rates. Therefore, we believe that the vaginal and cervical stimulation associated with TC intrauterine AI in sheep, rather than the TC AI catheter per se, was the critical factor that limited the number of ewes that lambed. Our future research will address hypotheses related to the potential effect of vaginal and cervical stimulation on embryonic mortality.

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